Amendments to the Specification

Please replace the paragraph beginning at page 1, line 8, with the following amended paragraph.

This application <u>is a divisional of Serial No. 09/632,553, filed 4 August 2000,</u> <u>which</u> claims the benefit of U.S. Provisional Application Serial No. 60/147,117, filed 4 August 1999, <u>both of</u> which ~~is~~ <u>are</u> incorporated herein by reference in ~~its entirety~~ <u>their entireties</u>.

Please replace the paragraph beginning at page 9, line 13, with the following amended paragraph.

Figure 2 <u>and 2A-1 through 2A-55 list</u> ~~lists~~ the atomic structure coordinates for recombinant *S. aureus* thymidylate kinase (with a His_6 tag) as derived by x-ray diffraction from a crystal of that complex. The following abbreviations are used in Figure 2:

Please replace the paragraph beginning at page 10, line 12, with the following amended paragraph.

Figure 3 depicts *S. aureus* thymidylate kinase using ~~(a)~~ a ribbon diagram showing the backbone structure of the enzyme <u>(3a)</u> and ~~(b)~~ a schematic diagram showing the secondary structure for a TMK monomer <u>(3b)</u>. Disordered loops are indicated by arrows.

Please replace the paragraph beginning at page 10, line 20, with the following amended paragraph.

Figure 5 depicts ~~(a)~~ a stereo view of a superposition of *S. aureus* thymidylate kinase and *E. coli* thymidylate kinase <u>(5a)</u> and ~~(b)~~ the amino acid sequence alignment of *S. aureus* thymidylate kinase (SEQ ID NO:1) (capital letters, upper sequence) and *E. coli* thymidylate kinase (SEQ ID NO:2) (lower sequence)<u>(5b)</u>. Dots in the sequences indicate gaps inserted in order to optimize the alignment. Identical residues are indicated by | and similar residues are indicated by . and : symbols.

Please replace the paragraph beginning at page 10, line 26, with the following amended paragraph.

Figure 6 depicts ~~(a)~~ a stereo view of a superposition of *S. aureus* thymidylate kinase and *S. cerevisiae* thymidylate kinase (6a) and ~~(b)~~ the sequence alignment of *S. aureus* thymidylate kinase (SEQ ID NO:1) (capital letters, upper sequence) and *S. cerevisiae* thymidylate kinase (SEQ ID NO:3) (lower sequence) (6b). Dots in the sequences indicate gaps inserted in order to optimize the alignment. Identical residues are indicated by | and similar residues are indicated by . and : symbols.

Please replace the paragraph beginning at page 11, line 4, with the following amended paragraph.

Figure 7 depicts ~~a)~~ a substrate-based inhibitor (AP_5T) for thymidylate kinase with a K_d of 20 nM for *E. coli* TMK (A. Lavie et al., <u>Biochemistry</u> 37:3677-86 (1998); A. Lavie et al., <u>Proc. Natl. Acad. Sci. USA</u>, 95:14045-50 (1998)) (7a)[[. b)]] and protein ligand interactions for *E. coli* TMK (shaded boxes, from A. Lavie et al., <u>Proc. Natl. Acad. Sci. USA</u>, 95:14045-50 (1998)) with the corresponding residues from *S. aureus* TMK underlined (conservative mutations are marked with an asterisk) (7b). Active site residues from the *S. cerevisiae* are boxed (where no corresponding residue from *E. coli* TMK is present, an arrow indicates the point of contact with the substrate).

Please replace the paragraph beginning at page 11, line 13, with the following amended paragraph.

Figure 8 depicts the anomalous difference Patterson maps at ~~(a)~~ 2.7 Å (8a) and ~~(b)~~ at 2.3 Å resolution (8b).

Preliminary Amendment
Applicant(s): Timothy E. Benson
Serial No.: Unassigned (Parent: 09/632,553)
Filed: Herewith (Parent: August 4, 2000)
For: CRYSTALLIZATION AND STRUCTURE DETERMINATION OF STAPHYLOCOCCUS AUREUS
THYMIDYLATE KINASE

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Please replace the paragraph beginning at page 11, line 15, with the following amended paragraph.

Figure 9 depicts electron density maps of residues 76 to 82 from molecule 1 of *S. aureus* thymidylate kinase (SEQ ID NO:1) at ~~(a)~~ 2.7 Å (9a) and ~~(b)~~ at 2.3 Å resolution (9b).

Please replace the paragraph beginning at page 11, line 18, with the following amended paragraph.

Figure 10 and 10A-1 through 10A-151 list ~~lists~~ the structure factors and multiple anomalous dispersion phases for the crystal structure of *S. aureus* thymidylate kinase (SEQ ID NO:1). "INDE" refers to the indices h, k, and l (columns 2, 3, and 4 respectively) of the lattice planes. "FOBS" refers to the structure factor (F) of the observed reflections. "SIGMA" is the standard deviation for the observations. "PHAS" refers to the phase used for the observations. "FOM" refers to the figure of merit.

Please replace the paragraph beginning at page 11, line 24, with the following amended paragraph.

Figure 11 depicts a surface representation of ~~a)~~ *E. coli* TMK with the inhibitor AP_5T (11a) and ~~b)~~ *S. aureus* TMK with a hypothetical positioning of AP_5T based on a structural alignment of C_α atoms from the *E. coli* TMK + AP_5T structure (11b).

Please replace the paragraph beginning at page 24, line 25, with the following amended paragraph.

The structure coordinates set forth in Figure 2 can be used to aid in obtaining structural information about another crystallized molecule or molecular complex. The method of the invention allows determination of at least a portion of the three-dimensional structure of molecules or molecular complexes which contain one or more structural features that are similar to structural features of *S. aureus* thymidylate kinase[[,]]. These molecules are referred to herein

as "structurally homologous" to *S. aureus* thymidylate kinase, Similar structural features can

include, for example, regions of amino acid identity, conserved active site or binding site motifs,

and similarly arranged secondary structural elements (e.g., α helices and β sheets). Optionally,

structural homology is determined by aligning the residues of the two amino acid sequences to

optimize the number of identical amino acids along the lengths of their sequences; gaps in either

or both sequences are permitted in making the alignment in order to optimize the number of

identical amino acids, although the amino acids in each sequence must nonetheless remain in

their proper order. Preferably, two amino acid sequences are compared using the Blastp

program, version 2.0.9, of the BLAST 2 search algorithm, as described by ~~Tatiana~~Tatusova et

al., FEMS Microbiol Lett 174, 247-50 (1999), and available from the world wide web at

~~http://www.~~ncbi.nlm.nih.gov/gorf/bl2.html. Preferably, the default values for all BLAST 2

search parameters are used, including matrix = BLOSUM62; open gap penalty = 11, extension

gap penalty = 1, gap x_dropoff = 50, expect = 10, wordsize = 3, and filter on. In the comparison

of two amino acid sequences using the BLAST search algorithm, structural similarity is referred

to as "identity." Preferably, a structurally homologous molecule is a protein that has an amino

acid sequence sharing at least 65% identity with a native or recombinant amino acid sequence of

S. aureus thymidylate kinase (for example, SEQ ID NO:1). More preferably, a protein that is

structurally homologous to *S. aureus* thymidylate kinase includes at least one contiguous stretch

of at least 50 amino acids that shares at least 80% amino acid sequence identity with the

analogous portion of the native or recombinant *S. aureus* thymidylate kinase (for example, SEQ

ID NO:1). Methods for generating structural information about the structurally homologous

molecule or molecular complex are well-known and include, for example, molecular

replacement techniques.

Please replace the paragraph beginning at page 36, line 13, with the following amended paragraph.

Specific computer software is available in the art to evaluate compound deformation energy and electrostatic interactions. Examples of programs designed for such uses include: Gaussian 94, revision C (M.J. Frisch, Gaussian, Inc., Pittsburgh, PA [[8]]1995); AMBER, version 4.1 (P.A. Kollman, University of California at San Francisco, [[8]]1995); QUANTA/CHARMM (Molecular Simulations, Inc., San Diego, CA [[8]]1995); Insight II/Discover (Molecular Simulations, Inc., San Diego, CA [[8]]1995); DelPhi (Molecular Simulations, Inc., San Diego, CA [[8]]1995); and AMSOL (Quantum Chemistry Program Exchange, Indiana University). These programs may be implemented, for instance, using a Silicon Graphics workstation such as an Indigo^2 with "IMPACT" graphics. Other hardware systems and software packages will be known to those skilled in the art.